and the beta-strands are important kinesin building blocks that act in concert during mechanotransduction. This work was funded by NIH (GM097350 to SK).

3935-Pos Board B663
Photo-Regulation of Mitosis Kinesin Kif18A using Photochromic Inhibitor
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Kif18A is a member of the kinesin-8 family was identified as a central component for the correct alignment of chromosomes at the spindle equator. Recent in vitro analyses revealed that Kif18A has a unique dual functionality, motility and depolymerase. Previously, BTB-1 was found to be the first small molecule inhibitor of Kif18A. BTB-1 potently inhibits the ATPase activity of Kif18A (IC50=1.69 μm) but not of other tested key mitotic kinesins. BTB-1 blocks the motility of Kif18A in a reversible manner. BTB-1 inhibits Kif18A in an adenosine triphosphate (ATP)-competitive but microtubule-uncompetitive manner and slows down the progression of cells through mitosis. In our previous study, we demonstrated that the conventional kinesin in which functional sites modified with azobenzene derivative exhibit photo-reversible alteration of ATPase activity accompanied by cis-trans photoisomerization of azobenzene. Interestingly, the backbone structure of cis-azobenzene resembles BTB-1. In this study, we designed and synthesized photochromic BTB-1 analogues composed of azobenzene derivatives in order to regulate ATPase and motor activity of Kif18A in the photo reversible manner. 2-nitro-4-chloro-azobenzene (NCAB) is one of the photochromic BTB-1 analogue we have synthesized, exhibited photo-reversible inhibition for the ATPase activity of Kif18A. We also examined the photo reversible effect of NCBA for the ATPase kinetics of Kif18A utilizing the FRET between fluorescent ATP analogue Mant-ATP and the Trp residue of Kif18A mutant F329W. Furthermore, we tried to synthesize BTB-1 analogue that have a photo-crosslinkable azido group for the purpose of photoaffinity labeling to identify the BTB-1 binding site on Kif18A.

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Photocontrol of Mitotic Kinesin Eg5 by Incorporating of Photochromic Molecule into the Functional Loop L5
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All member of kinesin superfamily contain a structurally conserved loop L5 near the ATP binding site. The length of the L5 vary among kinesin superfamily members. It is believed that L5 of kinesin is important region for motor function. Interestingly mitotic kinesin Eg5 has the longest L5 in comparing with other kinesins. The kinesin Eg5 is a microtubule plus-end directed homotetrameric molecular motor that is essential for the formation of a bipolar spindle during eukaryotic cell division. It has been demonstrated that L5 of Eg5 performed as a stabilizer for the Eg5-specific inhibitors (STLC, monastrol) complexes. These inhibitors bind to the same pocket on the Eg5 motor domain composed by of loop L5, α2 and α3. In this study, we tried to photo control of Eg5 ATPase activity by incorporating of photochromic molecule into L5. We prepared 5 mutants of Eg5 which have a single cysteine in L5 in order to incorporate thiol-reactive photochromic molecules. We also synthesized thiol-reactive azobenzene derivative, iodoacetyl-trity-azobenzene (IATAB) and spiropyran derivative, iodoacetyl-spiropyran (IASP). Azobenzene is photo-isomerized to trans form of hydrophobic by visible light irradiation, and to cis form of hydrophilic by UV light irradiation. Spiropyran is photoisomerized to zwitterionized merocyanine by UV light irradiation. Merocyanine is converted to hydrophobic spiropyran by visible light irradiation. Photochromic molecules were incorporated into the mutants stoichiometrically. The Eg5 mutant W127C and D130C modified with IASP showed reversible alteration of microtubule dependent ATPase activity upon UV and visible light irradiations. In addition, we also succeed in photo control of inhibitory effect of IASP-W127C and IASP-D130C by STLC.

3937-Pos Board B665
The Kinesin-1 Gating Mechanism Studied by Pre-Steady State Kinetics
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Kinesin-1 transports cargo along a microtubule by the hand-over-hand motion of its two motor domains. The ATPase cycles of the two motor domains (also termed "heads") are thought to be coordinated by a tension-sensitive transition(s). The "front head gating model" proposes that ATP binding to the front head is inhibited until the rear head dissociates from the microtubule, while the "rear head gating model" proposes that ADP release from the rear head is inhibited until this head dissociates and is pulled forward by the front head. Here we have tested these models by engineering a kinesin heterodimer that mimics a two-head-bound intermediate in the stepping cycle. Stop flow kinetic measurements were performed with mant-dATP and a FRET-based assay was used to distinguish nucleotide binding between the front and rear motor heads. Unexpectedly, we found that the rates of nucleotide binding to the front and rear microtubule-bound heads were similar (and similar to a kinesin monomer), which suggests that "gating" does not occur at the step of nucleotide binding. Our preliminary results suggest that phosphate release kinetics, as measured by phosphate binding protein, in the front head are slower in this microtubule-bound kinesin dimer. This study provides new insights into the kinetics steps that are involved in kinesin gating.

3938-Pos Board B666
Enhanced Transverse Motion of Multiple Kinesin Motor Configurations via a Diffusive Weakly Bound State
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Kinesins are a class of active molecular motors which mainly walk along microtubules from the negative to positive end in a highly processive manner and are regarded as tracking single protofilaments to a large extent. However, our experimental measurements of multiple-motor configurations reveal significant side-stepping motion without the loss of processivity. Here, we combine experimental data and theoretical modeling to demonstrate that, in two-motor configurations, one of the motors can be in a weakly interacting state that diffuses along the microtubule surface. At low ATP concentrations of 10 micromolar ATP we find double motor configurations to consist of mainly a single active motor together with a passively diffusing weakly bound motor. As the ATP concentration is increased to the saturating range, processivity decreases and configurations consist mainly of single active motors with unbound partners. We present a simple stochastic state transition model between actively engaged, diffusive weakly bound, and unbound states that can reproduce experimental results regarding ATP dependence and characteristics of transverse motion. Although the tethering effect of a weakly interacting motor acting to increase processivity has been studied before, we present novel measurements of the diffusive weakly interacting motor's diffusion across the microtubule surface and the resulting enhanced transverse motion of cargo. Functionally this enhanced transverse motion could be important for providing the flexibility needed for multiple motors to navigate a cargo through a crowded cellular environment.

3939-Pos Board B667
Kinesin-2's Neck-Linker is Critical to Navigating Obstacles on the Microtubule Surface More Efficiently Than Kinesin-1
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A number of cargo during intracellular transport are known to be bound to both kinesin-1 and kinesin-2, but the advantage of having two similarly plus-end directed motors on a single cargo is not clear. Kinesin-1 is known to be sensitive to alterations in the microtubule track, including those arising from post-translational modifications, changes in nucleotide state, and the presence of microtubule associated proteins (MAPs) such as Tau. Less is known about effects of microtubule lattice modifications on kinesin-2 motility. Kinesin-2, which contains three additional amino acids in its neck-linker compared with kinesin-1, has reduced stepping coordination between motor domains, which decreases its processivity on paclitaxel-stabilized microtubules. We hypothesize these differences in kinesin-2's structure and function allows it to more easily navigate obstacles on the microtubule surface, such as Tau, compared to kinesin-1. To directly test this hypothesis, we used single molecule imaging with TIRF microscopy to measure motility from different kinesin-1 and kinesin-2 neck-linker chimeras stepping along microtubules in the absence or presence of two isoforms of Tau known to differentially affect kinesin-1 motility. Our results demonstrate that kinesin-2, unlike kinesin-1, is insensitive in the presence of either Tau isoform on paclitaxel-stabilized microtubules. Swapping the neck-linkers between kinesin-1 and kinesin-2 resulted in a switch in the sensitivity to Tau between the two motors: the kinesin-1 construct containing a kinesin-2 neck-linker became insensitive to Tau, while the kinesin-2 construct containing a kinesin-1 neck-linker became sensitized to the presence of Tau. Thus, while kinesin-2 is less processive than kinesin-1, it is better optimized through its longer neck-linker to navigate obstacles on the microtubule surface, such as Tau, allowing the two motors to work together for the efficient delivery of cargo in the complex intracellular environment.